ISSN (print) 0093-4666 © 2012. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/122.389

Volume 122, pp. 389–398 *October–December 2012*

Synonymy of *Suillus imitatus*, the imitator of two species within the *S. caerulescens/ponderosus* complex

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Abstract—Identification of three species of *Suillus*, *S. caerulescens*, *S. ponderosus*, and *S. imitatus*, has always been difficult because of overlapping and non-discrete morphological characters. To solidify the identification of these taxa, we compared the nucleotide sequences from the internal transcribed spacer region (ITS) of the type specimens of *S. caerulescens*, *S. ponderosus*, *S. imitatus* var. *imitatus*, and *S. imitatus* var. *viridescens* with field collected specimens which we identified as *S. caerulescens, S. ponderosus,* and *S. imitatus* in northern California. Based on ITS sequence identity and phylogenetic inference, specimens of *S. caerulescens* and *S. ponderosus* formed well-supported clades with the holotype of the respective species. However, *S. imitatus* var. *imitatus* fits within the *S. caerulescens* clade and *S. imitatus* var. *viridescens* fits within the *S. ponderosus* clade. Therefore, we synonymize *S. imitatus* var. *imitatus* with *S. caerulescens* and *S. imitatus* var. *viridescens* with *S. ponderosus,* and show that the species can indeed be recognized morphologically based on annulus characteristics.

Key words—bolete, phylogenetics

Introduction

Suillus Gray is a large ectomycorrhizal genus in the *Boletales* with about 170 known species (Index Fungorum and Mycobank); about half of those species are found in North America (Bessette et al. 2000) and about 40 species in the western United States (Smith 1979). The four taxa in this study, *S. caerulescens*, *S. ponderosus*, *S. imitatus* var. *imitatus,* and *S. imitatus* var. *viridescens* (all within section *Boletinus*), are restricted to the North American west coast. Smith & Thiers (1964) described *S. caerulescens, S. ponderosus,* and *S. imitatus* as three distinct species based mainly on texture of the annulus/annulus remnant and pileus color. Later, Smith & Trappe (1972) described *S. imitatus* var. *viridescens* from western Oregon based on the strong greening reactions of cut tissue in the

Table 1. Morphological comparisons of four *Suillus* taxa. *

	S. caerulescens	*S. ponderosus*	*S. imitatus* var. *imitatus*	*S. imitatus* var. *viridescens*
Holotype	MICH12297 (Smith 48733)	MICH12305 (Smith 20204)	MICH12300 (Smith 48732)	MICH12301 (Smith 78754)
Pileus size	6–14 cm	9–25 cm	4–12 cm	4–12(–15) cm
Pileus color	Vinaceous, ochraceous tawny to ochraceous buff	Deep vinaceous brown to testaceous	Orange cinnamon to dingy cinnamon	Reddish orange, ferruginous, dull to cinnamon rufous to cream-buff
Tube mouths (mm)	1–2	1–3	2–3	~ 1
Stipe size (cm)	2.5–8 × 2–3	9–14 × 3–6	3.5–6 × 1.5–2.5	3–9 × 1–3
Stipe staining	Base slowly blue when cut	Surface blue to greenish	Base surface (1/2–1/3) quickly blue when cut	Surface blue and/or green in moist (not dry) specimen
Annulus	Band-like, fibrillose, pallid to white, not gelatinous	Membranous, reddish-cinnamon, gelatinous	Band-like, dingy pallid, staining brownish, felty-tomentose (edges with some gluten)	Wide band with buff-orange slimy edge, fragmentary in age
Spores (as reported)	8–11 × 3–5 µm	8–10(–12) × 3.8–5 µm	7–9 × 4–4.5 µm	8–11 × 4–4.5 µm
Spores (as measured)	7.6–10.2 × 4–4.4 µm	6–8.4 × 4–4.2 µm	7–10 × 4–4.5 µm	7–11 × 4–4.4 µm

* Adapted from Smith (1979), Smith & Thiers (1964), and Smith & Trappe 1972. Spore dimensions are reported based on the literature and as measured in this study.

stipe and the strong green colored specimens in the field. Table 1 provides an overview of the morphological characteristics of these four taxa.

The four taxa are recognized in monographs and western guidebooks (e.g. Arora 1986, Bessette et al. 2000, Smith & Thiers 1964, Thiers 1967, 1975). *Suillus caerulescens* and *S. ponderosus* are very common mycorrhizal associates of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), while *S. imitatus* was described as a spruce associate (Smith & Thiers 1964) and is listed in modern field guides as occurring in mixed conifer forests in the Pacific Northwest (Bessette et al. 2000). In many years, fruit bodies belonging to the *caerulescens/ponderosus* complex can be found in great abundance in coastal California forests. As a result, it is not unusual to come across these taxa and to be faced with the challenge of identifying them correctly. In the field, we rely heavily on annulus characters to differentiate between species (Fig. 1), but this is a short-lived, developmental character that can be altered or at least obscured by age

Figure 1. *Suillus caerulescens* (A) and *S. ponderosus* (B): Comparison of veil and other morphological characters.

and weather. Prof. Harry Thiers used to joke that in dry weather all specimens had a dry annulus and were called *S. caerulescens* and in wet weather the annuli were more gelatinous and the species was called *S. ponderosus*.

In contrast, the name *S. imitatus* is rarely applied to California material, except to those collections that have a greenish tint and are referred to as *S. imitatus* var. *viridescens* (Arora 1986). The original description of *S. imitatus* var. *imitatus* compared it primarily with *S. lakei* (Murrill) A.H. Sm. & Thiers, but field guides usually compare it with *S. caerulescens* from which it is distinguished with difficulty, by its supposedly slightly shorter spores and slightly more orangish pileus (Thiers 1975, Arora 1986, Bessette et al. 2000). *Suillus lakei,* another Douglas-fir associate closely related to the *caerulescens/ponderosus* complex (Kretzer et al. 1996), is easily distinguished by its reddish, fibrillose pileus. To help sort out the species present in our area, we combined existing GenBank ITS sequences with newly acquired ITS sequences from the holotypes and multiple collections of *caerulescens/ponderosus* complex.

Materials & methods

Our field collections were obtained from northern California (Table 2). We obtained distribution information from our field-collected specimens, those deposited in the University and Jepson Herbarium at the University of California, Berkeley (UC), photos deposited in the online database Mushroom Observer that have clear identification, and available checklists for Arizona (Bates 2006), Montana (C. Cripps pers. comm.), and the Intermountain Region (Piep 2003). These sources combined to provided an acceptable account for the overall natural distribution of the species studied here.

We extracted DNA from freshly collected specimens (UC 1861375-UC 1861389) using a modified Sigma Extract-N-Amp kit (Sigma Aldrich, St. Louis, MO). For

Table 2. Specimens sequenced in this study.

Species	Collection date	Collection location*	Herbarium^ #	GenBank #
S. caerulescens	3 Dec. 2006	CA: San Francisco Bay area	UC 1861383	JQ958308
	3 Dec. 2006	CA: San Francisco Bay area	UC 1861384	JQ958309
	3 Dec. 2006	CA: San Francisco Bay area	UC 1861385	JQ958310
	3 Dec. 2006	CA: Marin Co., Mill Valley, Mt. Tamalpais	UC 1861386	JQ958311
	3 Dec. 2006	CA: Marin Co., Mill Valley, Mt. Tamalpais	UC 1861387	JQ958312
	3 Dec. 2006	CA: Marin Co., Mill Valley, Mt. Tamalpais	UC 1861388	JQ958313
	3 Dec. 2006	CA: Marin Co., Mill Valley, Mt. Tamalpais	UC 1861389	JQ958314
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861378	JQ958315
S. ponderosus	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861375	JQ958320
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861376	JQ958321
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861377	JQ958322
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861379	JQ958323
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861380	JQ958324
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861381	JQ958325
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861382	JQ958326
S. caerulescens, holotype	9 Oct. 1954	WA: Pierce Co, Spanaway Park	MICH 12297	JQ958316
S. imitatus var. *imitatus,* holotype	9 Oct. 1954	WA: Pierce Co., Mt. Rainier National Park, Power House Woods	MICH 12300	JQ958317
S. imitatus var. *viridescens,* holotype	2 Oct. 1970	OR: Lincoln Co., near Otis, Van Duzer Corridor	MICH 12301	JQ958319
S. ponderosus, holotype	24 Oct. 1944	OR: Clackamas Co., Rhododendron	MICH 12305	JQ958318

*Location abbreviations: JDSF = Jackson Demonstration State Forest.

^Herbarium codes: UC = University and Jepson Herbaria, University of California, Berkeley; MICH = University of Michigan Herbarium.

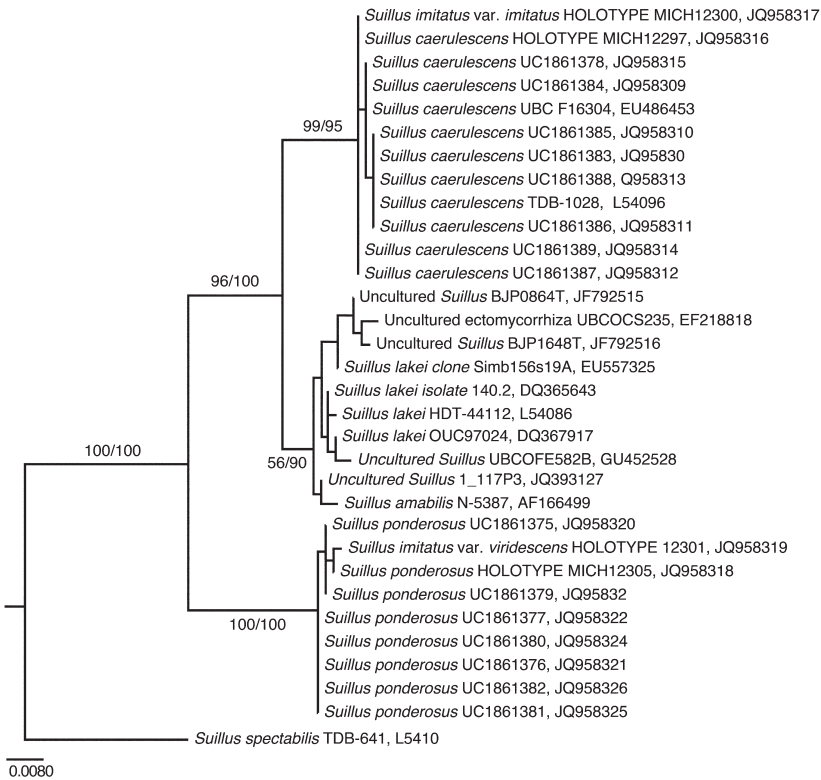

Figure. 2. ITS-sequence based maximum likelihood tree of the *Suillus* species in this study. Maximum likelihood followed by maximum parsimony bootstraps provide statistical support for branches with >50% support. Each sequence contains the isolate/specimen number followed by a GenBank accession number. *Suillus spectabilis* served as the outgroup.

specimens that had been stored in a herbarium, it was necessary to obtain high quality DNA so we used a standard CTAB buffer lysis followed by chloroform extraction and ethanol precipitation. DNA was extracted from the following herbarium type specimens: MICH 12305, MICH 12297, MICH 12300 and MICH 12301. Herbarium abbreviations are according to Thiers (2012; continuously updated). For field/recently collected specimens, we used the standard fungal specific primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). For herbarium specimens with degraded DNA we amplified the ITS gene in two segments when necessary. We used standard ITS and *Suillus* ITS specific primers (ITS-2S and ITS-3S) in various combinations: ITS1F and ITS2S or ITS2 and ITS3S or ITS3 and ITS4 (Bruns et al. 2010). Sequences were produced using the standard BigDye Terminator v3.1 Kit and ran on an ABI Prism 3700 Genetic Analyzer (Life Technologies).

We examined each automated sequence and manually interpreted and corrected ambiguous bases using Sequencher (Gene Codes Corporation). These sequences were deposited in GenBank under accession numbers (JQ958308-JQ958326). Using BLAST we gathered from GenBank all sequences similar to the species mentioned as of April 24, 2012: *S. lakei* (GenBank L54086, DQ365643, DQ367917, EF218818, AF166499, GU452528, JF792516, EU557325, JQ393127, JF792515), *S. caerulescens* (GenBank L54096, EU486453), and *S. spectabilis* (Peck) Kuntze (GenBank L54104) was used as an outgroup species. We aligned the sequences manually and analyzed the alignment using the maximum likelihood model GTRGAMMA in RAxML (Stamatakis 2006). Maximum likelihood bootstrap was performed using the RAxML online BlackBox interface (Stamatakis et al. 2008). Maximum parsimony bootstrap was performed using PAUP v.4.0b6 with 1000 replicates (Swofford 2001). Corrected distances between sequences were found using the Kimura 2-parameter in PAUP (Swofford 2001).

Results & discussion

We obtained full length ITS coverage for three of the four type specimens along with the 15 specimens that we collected for this study. We were able to obtain only the ITS1 and ITS2 regions of the *S. caerulescens* holotype MICH12297. We combined the sequences generated with GenBank sequences of *S. lakei*, and the outgroup sequence from *S. spectabilis*. The matrix contained 32 taxa, 613 characters, and 30 parsimony informative characters. The resulting maximum likelihood tree is shown in Fig. 2.

To our surprise specimens that we had determined as *S. caerulescens* and *S. ponderosus* based on annulus differences (Fig. 1) separated into two clades with good statistical support (Fig. 2). Despite the morphological similarities between these two species, *S. caerulescens* is sister not to *S. ponderosus,* but to *S. lakei*. Together these three species form a clade with moderately high support (85%) based on an analysis of 40 *Suillus* taxa (data not shown). From these results, we conclude that annular characters, when present, are a reliable way to separate *S. caerulescens* from *S. ponderosus* (Table 1, Fig. 2).

In contrast, *S. imitatus* sequences did not fall within their own clade but fit within the clades for the two other species: *Suillus imitatus* var. *imitatus* within the *S. caerulescens* clade, and *S. imitatus* var. *viridescens* within the *S. ponderosus* clade. At least so far, morphologically defined *Suillus* species separate fairly well by ITS, but we know from other taxa that not all species may be distinguished by differences within the ITS region (Manian et al. 2001, Bruns et al. 2010). This prompts the question of whether there are morphological reasons to suspect that one or both *S. imitatus* varieties might be distinct at the species level. Interestingly, in the discussion that followed separation of the *S. imitatus* varieties, Smith & Thiers (1964) and Smith & Trappe (1972) initially thought that *S. imitatus* var. *imitatus* might represent *S. caerulescens* and *S. imitatus* var. *viridescens* might be *S. ponderosus*. They decided instead to recognize two

taxa separated by pileus color and spore size differences. The shorter spores for *S. imitatus* var. *imitatus* reported by Smith & Thiers (1964) and cited in many later references were given as 7–9 × 4–4.5 µm. Although this overlaps the lower end of the spore range given for *S. caerulescens* (8–11 × 3–5 µm), it could indicate a subtle distinction. However, we re-measured the spores of the type collection as 7–10 × 4–4.5 µm, which now puts the var. *imitatus* spores within the center of the range for *S. caerulescens, S. ponderosus,* and *S. imitatus* var. *viridescens.* The orangish pileus color of *S. imitatus* var. *imitatus* certainly overlaps the highly plastic pileus colors seen in *S. caerulescens* and *S. ponderosus,* and the greenish tint of *S. imitatus* var. *viridescens* could easily be environmentally induced, much as the aqua colors seen in the stipe of *Leccinum* species (den Bakker & Noordeloos 2005). For these reasons we see no compelling argument to maintain either variety of *S. imitatus* as a separate taxon and so synonymize var. *imitatus* with *S. caerulescens* and var. *virescens* with *S. ponderosus*.

Suillus lithocarpi-sequoiae was described by Singer (1960) from Muir Woods, Marin County, California. The descriptions for this species fit well within our current concept of *S. ponderosus*. The type specimen (Singer N 1531) was deposited in Fundación Miguel Lillo, Argentina (LIL). Unfortunately, the type specimen could not be located and is assumed lost. Singer placed (1962) and maintained (1975, 1986) this species in *Pulveroboletus,* even though Smith & Thiers (1964) and Thiers (1975) thought it better placed in *Suillus*. The species was never encountered again at the type locality, despite repeated search efforts (Thiers 1975). For these reasons we consider the name *S. lithocarpi-sequoiae* doubtful.

Below is an updated taxonomy, and emended diagnoses for *S. caerulescens* and *S. ponderosus*.

Taxonomy

Suillus caerulescens A.H. Sm. & Thiers, Contr. Monogr. N. Amer. *Suillus*: 36, 1964. FIGURE 1A

HOLOTYPE: MICH12297 (A.H. Smith 48733); GENBANK nrITS JQ958316.

= *Suillus imitatus* A.H. Sm. & Thiers var. *imitatus*, Contr. Monogr. N. Amer. *Suillus*: 40, 1964.
HOLOTYPE: MICH 12300 (A.H. Smith 48732); GENBANK nrITS JQ958317

These two names were validly published at the same time and therefore have equal priority. However, we have chosen to retain the name *S. caerulescens* over *S. imitatus* because of its broader use in many research articles, guidebooks, and Internet web pages.

CHARACTERISTICS—Annulus band-like, fibrillose to felty-tomentose, white to dingy pallid, staining brownish, not gelatinous to slightly gelatinous with some gluten along the edges.

Suillus ponderosus A.H. Sm. & Thiers, Contr. Monogr. N. Amer. *Suillus*: 38, 1964. FIGURE 1B

HOLOTYPE: MICH12305 (A.H. Smith 20204); GENBANK nrITS JQ958318

= *Suillus imitatus* var. *viridescens* A.H. Sm. & Trappe, Mycologia 64: 1151, 1972.

HOLOTYPE: MICH12301 (A.H. Smith 78754); GENBANK nrITS JQ958319

CHARACTERISTICS—Annulus membranous and band-like, buff-orange to reddish-cinnamon, gelatinous to highly gelatinous and slimy along the edge.

COMMENTS— Both species seem to occur within the host range of Douglas fir (*P. menziesii*). *Suillus caerulescens* occurs from coastal northern California north to British Columbia (Canada) and Montana, and in Arizona. It has also been found in the northern Sierra Nevada at lower elevations where *P. menziesii* occurs (E.C. Vellinga pers. obs.). There is one report of its occurrence in northern San Diego County, CA, on Palomar Mountain (Mushroom Observer 2012: 61662). However, *Pseudotsuga macrocarpa* (Vasey) Mayr rather than *P. menziesii* occurs there. Unfortunately this material was not available for study. *Suillus ponderosus* occurs in coastal areas from northern California (north of Monterey Bay) to northern Oregon, but it is unclear whether it occurs in the Sierra Nevada. *Suillus lakei,* sister to *S. caerulescens*, occurs in coastal northern California, north into Montana, British Columbia, and Alberta (Canada) and in patchy areas of the Rocky Mountains in Colorado, Utah, New Mexico and Arizona (Mushroom Observer, Piep 2003, Bates 2006). This wide distributional range may explain the ITS variations seen in FIG. 2.

To understand ITS genetic variability within and among the three species in this study, we determined the intraspecific and interspecific percent sequence similarity based on the corrected Kimura 2-parameter. Together with the type specimens, all sequences within each of the three clades show high intraspecific similarity: *S. caerulescens* (99.8–100%), *S. ponderosus* (99.5–100%), *S. lakei* (98.4–100%). However, the interspecific similarity can be as high as 98.8% among species: *S. caerulescens* (93.8–98.8%), *S. ponderosus* (93.8–96.8%), *S. lakei* (94.3–98.8%). This is another case in which strict ITS similarity [the 97% sequence similarity that serves as a proxy for operational taxonomic unit (OTU) separation in ecological studies] does not necessarily correctly differentiate species. Further study is necessary to determine the ITS genetic variability for the whole genus *Suillus*. However, based just on what we already observed here, caution is needed when using the broad implementation of the 97% unilateral separation for all taxa (reflected in Hibbett et al. 2011, and references therein). We strongly recommend that a phylogenetic framework be used for species delimitation in studies using DNA sequences.

Acknowledgements

We thank the Herbarium at the University of Michigan for providing loans of type specimens and the two reviewers, Boris Assyov and Dimitar Bojantchev, for their useful comments to improve the manuscript. Support for this research was provided by the NSF-GRFP to NHN and a Sonoma County Mycological Association (SOMA) Graduate Scholarship to JFK.

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